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PRIMER NOTE

Isolation and characterization of 10 microsatellite loci from *Iris hexagona* (Iridaceae)

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Abstract

Ten microsatellite loci isolated from *Iris hexagona* are described. All these loci are polymorphic, with three to 13 alleles across 24 individuals from a single natural population. Heterozygosity ranged from 0.125 to 0.870. Three loci depart significantly from Hardy–Weinberg equilibrium in our test population. The test population shows significant heterozygote deficiency in these and two other loci. Three loci exhibit significant linkage disequilibrium. These loci will be utilized to investigate patterns of genetic variation in the species throughout the Florida peninsula.

Keywords: *Iris*, Louisiana iris, microsatellite, population genetics, SSR

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Iris hexagona Walter is the only native iris species in peninsular Florida. It is a member of the series Hexagonae, a small complex of three to four species and numerous hybrid populations (Randolph 1966; Arnold *et al*. 1990; Arnold 1993). *Iris hexagona* occurs mostly in open, freshwater swamps in Texas, Louisiana, Mississippi, Alabama, Georgia, South Carolina, and Florida (Viosca 1935; Bennett 1989), though this broad range assumes synonymy of *Iris giganticaerulea* Small. *I. hexagona* achieves its broadest geographical range in Florida, occurring throughout the peninsula.

One of us (M.G.) has observed populations of this species throughout Florida, and has documented much broader variation in morphology and habitat than has been previously associated with this species. Our hypothesis is that populations of *I. hexagona* within any major Florida drainage system represent unique genetic assemblages that radiated from refugia during interglacial periods of inundation when Florida was reduced to a series of islands. We propose to test this hypothesis using microsatellite DNA markers developed from the genomic DNA of *I. hexagona*, as previous SSR isolations from other species in section Hexagonae have proven informative (Burke & Arnold 1999).

We isolated microsatellite loci from *I. hexagona* using a modification of the enrichment method of SSR marker development in Edwards *et al*. (1996). Genomic DNA was restricted, ligated to adaptors and amplified with polymerase chain reaction (PCR). The amplicons were hybridized twice with biotin-labelled synthetic SSRs and isolated using streptavidin coated beads (Dynal) in conjunction with a Dynal Magnetic Particle Concentrator. The eluted fragments were size separated using Sepharose CL-4B Size-Sep 400 Spun Columns (Amersham Pharmacia Biotech), amplified and cloned (using phage and plasmid vectors with M13 priming sites), and the clones screened by sequencing. Approximately 70% of our clones contained a repeat. Reverse sequences were obtained for these, and primers were designed from the flanking regions. The primers were first tested using labelled dNTPs on the genomic DNA from which they were isolated as well as a few individuals from other populations. If successful and polymorphic, a fluorescently labelled forward primer was subsequently obtained. Ten primer pairs (Table 1) were tested across a Florida population of *I. hexagona* (Levy County, 34 miles west of I-75 on SR 24 to Cedar Key). Differences in allele size were detected on an ABI 3100 Genetic Analyser (Applied Biosystems) using capillary gel electrophoresis. Reaction mix for primers at 50 °C annealing temperature was 1.0 μL 10 × buffer with 15 mM $MgCl_2$, 0.2 μL 10 mM dNTPs, 0.25 μL each forward and reverse primer, 0.05 μL *Taq* polymerase, 1.0 μL genomic DNA template, and 7.25 μL dH_2O for a total volume of 10 μL. For primers at 60 or 65 °C annealing temperatures, 1.0 μL of

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Table 1 Primer sequences and characteristics of 10 *Iris hexagona* microsatellite loci

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Repeat
IH42	AY822016	F: GAAGTTTTGTAGAGGTCTGGTG R: GCTACAACTAAAGAGTCCAGTC	$(AG)_{19}$
IH56	AY822021	F: AGCGGAAAGAATGAACACAAG R: GCGAAGAAGGAGCAAATAGTAG	$(CT)_{13}$
IH57	AY822023	F: TGAGAGAGAGACTGAGCATC R: TGATAGGAGGAGGGAGAGAAG	$(CT)_{31}$
IH63	AY822024	F: GCACATAACCTCTCTTGTCC R: ACCAGACTACGAATCATTACCC	$(TC)_{22}$
IH73	AY822018	F: TTCAAGCCTCTACTAGAGAGAC R: GCAACCTTGTAACCATCCC	$(TG)_{15} + (GTG)_5$
IH86	AY822022	F: TCAATCTTCTTTATCATGCAC R: TATGTATTCAATGCTTTGGAAC	$(TC)_{15}$
IH122	AY822025	F: TGGTCCTAAGTGACTGATAGTG R: GGAGTAGAGAGTGACATGGAG	$(TC)_{16}$
IH153	AY822017	F: GAGAAAGAAGGAGGAGGAAGG R: CAGCAACTGTGAGGAGAAAG	$(GA)_{21}$
IH155	AY822020	F: GTGCGAGATATAAGGGAAAGAC R: GATATTACCCATGCTAAGGCAG	$(GA)_{19}$
IH183	AY822019	F: TCTCAGGAATAGGGTGTGAC R: CACGAGCTAAATCACGATAGG	$(TGT)_{18}$

Locus	Annealing temperature (°C)	*n*	No. of alleles	Allele size range (bp)	Mean H_E	Mean H_O	f
IH42	50	22	3	123–149	0.665	0.840	−0.962
IH56	60	24	4	246–273	0.718	0.708	0.014
IH57	60	22	10	201–247	0.859	0.409*	0.530
IH63	50	23	13	90–118	0.878	0.870	0.010
IH73	50	24	3	167–173	0.196	0.125	0.367
IH86	50	24	4	134–143	0.593	0.542	0.088
IH122	65	17	8	85–104	0.674	0.588	0.130
IH153	50	22	6	207–226	0.703	0.455*	0.359
IH155	65	24	10	236–255	0.836	0.500*	0.407
IH183	50	24	7	138–180	0.634	0.750	−0.188
Mean			7.3		0.676	0.567	0.163

H_E, expected heterozygosity; H_O, observed heterozygosity; *f*, estimate of fixation index.
*Departs significantly from HWE at $P < 0.05$.

5 M betaine was substituted for 1.0 μL dH_2O. PCR program for all primers was 5 min at 94 °C, 35 cycles of 45 s at 94°C, 45 s at 50°C, 1 min at 72°C, 7 min at 72°C, and 4°C storage. Preliminary analysis of raw microsatellite data was performed using GENEMAPPER version 3.0 (Applied Biosystems). Descriptive statistics (Table 1) were generated with GDA version 1.1 (Lewis & Zaykin 2002). Tests for Hardy–Weinberg equilibrium (HWE) exact test and linkage disequilibrium (LD) were run with GENEPOP version 3.4 (Raymond & Rousset 1995).

None of the loci is monomorphic across the test population. Three loci (*IH57*, *IH153* and *IH155*) depart significantly from HWE ($P < 0.05$) in our test population. The test population shows significant heterozygote deficiency at these loci, and also at *IH73* and *IH122*. Whether this indicates the presence of null alleles in some of these loci is yet unknown. Heterozygote excess is only apparent in two loci, *IH42* and *IH183*. Three loci (*IH42*, *IH63*, and *IH183*) showed significant LD ($P < 0.05$).

The application of SSR data to *I. hexagona* will allow the discrimination of genetically related populations of the species, quantification of the levels of genetic variation within these populations, and patterns of gene flow among them. We are currently testing additional microsatellite primer pairs from our library of clones for their utility in these investigations.

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